27821 Course Schedule in short

In general: We will be running a total of 5 biofilm/flow cell experiments (ex 1- 5). Each team will be responsible for one flow cell system. This means that the team must assemble the system, sterilize it, and fill it with medium according to the instructions. Furthermore, the team must daily check that the medium supply is sufficient and empty the waste. All teams will inoculate and work with flow channels from all of the six systems, so the "responsibility" is limited to the above mentioned maintenance tasks. Teams that are going to inoculate strains in the free exercise biofilm system will also be responsible for media supply and waste in this system.
There is in addition one adhesion experiment and ond diagnostic exercise.
The free exercise is what the name implies: free. This means you can decide which experiments you want to perform and which analyses you want to do. The work on this exercise is administered almost entirely by yourself (there are, however, some time slots assigned to this). *Bring your own samples of interest for the free exercise or sample from the local area during the first part of the course.*

Friday 23/8 (Copenhagen University):

9:00 The exercises and practical information
Technical Talk: Janus Haagensen and Claus Sternberg: Biofilms and tools, confocal microscopy.
10:15 Coffee Break
10:30 Susanne Knøchel and NN: Intro to Adhesion exercise.
11:00 Eva Kammer Andresen and Janus Haagensen: Intro to ex 3.
11.30 Talk: Bitplane AG: Imaris software – what is possible?
12:00 Lunch.
1:00 Adhesion assay.
Gluing of flow chambers
The free exercise: Sampling of bacteria from your own chosen environment (contact lenses, soil, plants, foods items etc). Isolation of bacteria/plating and incubation at different temperatures.
Inoculation of already prepared and sterilized system 1 and 3 (done by teachers).
5:00 End of day 1

Saturday 24/8:

9:00 Talk: Lars Jelsbak and Lea Madsen: Bacterial evolution + Intro diag exercise
9:30 Claus Sternberg: intro to ex 1 and 2
10:00 Building Biofilm setups (All in all we will work on 5 systems during the course, each team of 3 will be responsible for 1 system with respect to media preparation and waste removal, all teams will work on all systems)
Medium preparation for all biofilm systems.
Confocal microscopy and image acquisition of the *E. coli* strains (biofilm system 1). Will take place most of the day one team at the time (images for COMSTAT).
12:00 Lunch.
1:00 Talk: TBD: TBD
2:00 Sterilization of biofilm system2, and 4/5.
Microtiter assay: inoculation of 2 plates, one that you will proceed with by hand and one that will be handled using a robot (*E. coli* and mutants, each team make their own plates).
The free exercise: Inspect plates and continue as you want from own ideas or guidelines in the manual material.
MIC determinations of *P. aeruginosa* determined by different conc. of colistin in test tubes and using E-test (have to be used later in the course for biofilm experiments (system 3)),
5:00 Rounding up in the lab with things you did not manage during the day
6:00 End of day 2

Sunday 25/8:

9:00 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).
Inoculation of biofilm system2, and 4/5 (if you have isolated strains you would like to see develop in a flow cell).
Start of diagnostic exercise (Multiplex PCR and plate prep)
Microtiter assay: Washing and reading (OD measurement and Crystal violet staining) as well as introduction to and starting up of the robot with your second microtiter plate.

12:00 Lunch.

1:00 Technical Talk: Claus Sternberg: FISH and COMSTAT

1:30 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).
Diag. exercise contd.
Look at MIC determinations tubes and plates.
Free exercise (continued… QS, make plates for motility assay, biofilm assays, microscopy/morphology structures on plates …).

6:00 End of day 3.

Monday 26/8:

9:00 Talk: Susanne Knøchel: Reemergence of bacterial contamination in food plants – are biofilms to blame?

10:00 COMSTAT on obtained images
Imaris treatment of images.
Diagnostic exercise: Antibiotic test. Hybridization/detection

12:00 Lunch.

1:00 **Talk: Per Væggemose: Hygiene and biofilms – a problem in food industry.**
Addition of antibiotics to biofilm system 3.
Diag. exercise contd.
Analyzing data from COMSTAT.
Work on the free exercise (continued…).

5:00 End of day 4.

Tuesday 27/8:

9:00 **Talk: Anders Folkesson: Biofilm formation and antibiotic tolerance: is there a connection?**

10:00 Staining of biofilm system 3 with Propidium Iodid (*P. aeruginosa* +/- antibiotics and *P. aeruginosa* (Yfp)+ *P. aeruginosa* pilA (Cfp))
Diagnostic exercise: Insepct plates. Data analysis.
Image acquisition of biofilms, system 3 continued during most of the day
Inspect biofilm system 2 before FISH plating and sorting tomorrow.

12:00 Lunch.

1:00 Image acquisition of biofilms, system 3 continued during most of the day
Diagnostic exercise contd.
Work on your free exercise (follow biofilm development if you have inoculated flow system 5 with your isolated strains)
Inspect biofilm system 2 before FISH, plating and sorting tomorrow.
Treat images using Imaris, COMSTAT,…

Wednesday 28/8

9:00 Lars Behrend, Microenvironmental analysis of biofilms – techniques and concepts

10:00 Embedding and hybridization+havesting and fixation and plating of biofilms (*P. aeruginosa* + *Acinetobacter*; biofilm system 2) (procedure continues throughout the whole day).
Read micro titer assay on Elisa reader
Work on the free exercise (continued…).
Diagnostic exercise: finishing.

12:00 Lunch.

1:00 Continue the work from biofilm system 2
Hybridzation contd.
Work on the free exercise (continued…).

6:00 End of day 6

7:30 Dinner (time and place tbd)

Thursday 29/8:

9:00 **Talk: Morten Alhede, Quorum Sensing and QS inhibition**

10:00 Image hybridized samples from biofilm system 2
FACS of hybridized single cells from biofilm system 2
Image acquisition of biofilm system 4/5

12:00 Lunch.

1:00 **Talk: Søren Molin TBD**

2:00 Image acquisition of biofilm system 4/5
Work on free exercise Data treatment, preparation of presentation.
Inspect the last samples. Treat images using Imaris.
Cleaning procedures for biofilm systems.

6:00 End of day 7.

Friday 30/8 (Copenhagen University):

9:00 The last preparations for the presentations

10:30 Presentation of results.

12:00 Lunch

1:00 Last presentations of results and evaluation.

4:00 End of course.

Biofilm Systems used.

Biofilm system 1: *E. coli* (traA), *E. coli* (F+), *E. coli* (traD) mutant, Image acquisition and COMSTAT
(15 channel system, Media FB with Fe-EDTA+A-10. Addition of glucose+proline+thiamine).

Biofilm system 2: *P. aeruginosa* (CY5 blue probe) + *Acinetobacter* (Cy3 red probe): FISH, directly on biofilm and syto staining of harvested cells. Also plating of harvested cells on selective plates (PA will grow on 100 µg/ml Amp) (ACN will grow on 100µg/ml Strep) + FACS sorting/counting for estimation of ratio between the species after biofilm establishment havesting and FISH.
(15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 3: *P. aeruginosa* (Gfp) alone two channels +/- antibiotic treatment and *P. aeruginosa* (Yfp) + *P. aeruginosa* pilA (Cfp) for structure development, image acquisition after antibiotic treatment.
(15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 4 and 5: Prepared for the free exercise (Participants own strains or strains isolated during the course). We will consider mediums dependent on the strains.

Time lapse will be shown as demonstration videos and explained during the CLSM sessions.